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ORIGINAL PAPER

Effect of seed treatment for laboratory germination of *Albizia chinensis*

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Abstract: Seeds of *Albizia chinensis*(Osb.) Merr. in addition to water were also treated with different treatments by incubating in ethyl alcohol, acetone, and petroleum ether at room temperature for different durations. Seed heat treatment was done at temperatures of 30, 40 and 60ºC for different durations up to 24 h. To overcome dormancy caused by the impermeable seed coat, seeds were nicked and also treated with concentrated sulphuric acid for different durations. Seeds responded to treatments with sulphuric acid and nicking only. Treatment with sulphuric acid for 20 and 30 min showed maximum germination at all incubation temperatures as compared to untreated controls and seeds treated with sulphuric acid for 10 min and nicking. Seedling length was greatest from seeds treated with sulphuric acid for 20 and 30 min and incubated at 30 ºC. Seedling dry weight was highest from nicked seeds incubated at 20ºC. The most favourable incubation temperature was 30 ºC as evidenced from G_{RI} (germination rate index) and G_V (germination value). After ascertaining the seed response and performance we recommend that seeds of *Albizia chinensis* be treated with sulphuric acid for 20 or 30 min and incubation temperature of 25 to 30ºC.

Keywords: Germination Rate Index; Germination Value; seed treatment

Introduction

Albizia chinensis(Osb.) Merr. is a tree species with a wide distribution in the Indo-Malayan region across a range of altitudes. Due to excessive felling of trees coupled with poor seed germination, its survival in the wild is affected. Therefore, this species is a candidate for conservation, particularly for *ex-situ* conservation in seed banks. For this strategy it is worthwhile to investigate its germination requirements and behavior under controlled laboratory conditions as pre-requisites for subsequent investigation of its storage behavior.

In this species, as in seeds of other members of Leguminosae coat-imposed dormancy is caused by the hard and thick seed coat, and germination percentage remains as high as 99% for periods up to 5 years when seeds are stored at cold temperature of 4ºC. The species is a nitrogen fixer, is drought resistant, provides shade, and is of commercial interest due to its fast growth.
The seeds develop in pods of about 7.5 cm × 1−2 cm. The number of seeds in each pod varies from 10-12 and each seed is circular in shape, flat, with a green seed coat, and seed diameter is about 0.5 cm.

Many species of the Leguminosae exhibit coat-imposed dormancy due to the hard seed coat which is impermeable to water and oxygen (Rolston 1978). Pre-treatment of seeds of tree legumes include cold or hot water treatment, dry seed treatment, manual or mechanical scarification, and acid scarification (Doran et al.. 1983; Willan 1985; Cruz et al. 1995; Gonzalez-Melero et al. 1997). Responses to seed treatment vary by species.

Germination requirements and behavior of seeds of *Albizia chinensis* have not been documented. This species was selected for our investigation because germination under natural conditions in the wild is negligible despite the fact that large number of seeds is shed each year. This is corroborated by experts in forest management. Based on poor germinability of seeds and excessive exploitation of this species in the wild, our study objective was to understand as how seed dormancy can be overcome by treatments. *A. chinensis* seeds are in high demand for forestry, agroforestry and commercial plantations and also for our subsequent investigation on seed behavior in storage.

Materials and methods

Seed collection

In March–April, 2007, clusters of pods of *A. chinensis* were collected from a single tree using a tree pruner. The pods were spread in a single layer and sun-dried for 3–4 days, after which the exposed seeds were manually collected, cleaned, and sealed in polythene bags. On arrival in our laboratory the seeds were

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kept in air tight polycarbonate containers and maintained at 20 ºC for 2 months, when temperature was reduced to 4ºC until our investigation on seed treatment for germination was performed in mid 2010.

Seed moisture determination

The seed moisture content was determined by drying seeds at 103±1ºC for 17 hours and the results were expressed as percentages of wet weight (ISTA 2008).

Germination test

Seeds were sterilized with 0.1% $HgCl_2$ followed by repeated washing with distilled water and then rolled in moistened paper towel. Other substrata such as sand and filter paper were used, but the best results were achieved with paper towel, hence soaked paper towel was used. The rolled paper towels were wrapped in waxed paper and then incubated in seed germinators maintained at 20, 25 and 30ºC. The score on radicle emergence (1 mm length) was compiled on alternate days and the final count was done on day 21. Seed performance was graded as normal, abnormal, dead and hard seeds (ISTA 2008). The secondary data derived from daily germinants like germination rate index (G_{RI}) and germination value (G_V) were calculated using different formulae prescribed by different workers as given below.

The germination rate index (G_{RI}; Maguire 1962) was calculated using the following formula:

$$G_{RI} = \sum \frac{N_i}{D_i} \quad (1)$$

where, N_i is the number of germinants(radicles) germinated on the day D_i counted from the day of sowing at a particular temperature and substratum. The Germination Value (G_V; Czabator 1962) was calculated using the following formula:

$$G_V = M_{DG} \times P_V \quad (2)$$

where, M_{DG} is the mean daily germination calculated as the percentage of germinated seeds at the end of the test divided by the number of days to the end of the test; P_V is the peak value or the maximum quotient derived from all of the cumulative germination percentages on any day divided by the number of days to reach this percentages.

The germination value, G_V, proposed by Djavanshir and Pourbeik 1976) based on emerged radicles of treated and untreated seeds was calculated using the following relation:

$$G_V = \sum \frac{D_{GS}}{N} \times G_P \times 10 \quad (3)$$

where, D_{GS} is the daily germination speed which is computed by dividing cumulative germination percentage by the number of days since the beginning of the test, N is the frequency or the number of D_{GS} that are calculated during the test' G_P is the germination percent at the test conclusion and is used in the formula as the number of germinated seeds over 100 and 10 is a constant.

Seed treatment

In addition to the water treatment, chemical seed treatment was also carried out by incubating seeds for 10, 20, and 30 min in ethyl alcohol, acetone and petroleum ether. Oven-dry-heat treatment of dry seeds was conducted by exposing seeds to 30, 40 and 60ºC for different durations up to 24 h. Seed coats were manually scarified (nicked) by removing a 1mm slice of the cotyledon distant from the hilum. For acid scarification, seeds were deep-soaked in concentrated sulphuric acid for 10, 20 and 30 min at room temperature, followed by washing in running tap water for about 30 min. All treated seeds were sterilized with 0.1% $HgCl_2$ solution followed by repeated washings with distilled water and put for germination in moistened rolled paper towel.

Seedling length and dry weight

After the seedling evaluation on day 21, we recorded the length of ten seedlings in three replicates and the mean of means was reported and expressed as cm per seedling. Ten seedlings in three replicates were dried at 90 ºC for 24 h and the mean of means of seedling dry weight was expressed as gm per seedling.

Statistical analysis

All primary and secondary data (G_{RI}, G_V) were subjected to ANOVA. Cumulative germination percentages were not transformed to arcsine square root. Differences between means for each parameter were tested (p =0.05) using least significant difference (lsd).

Results

Seed moisture was about 6.74% and the germination percentage of control seedlings ranged from 0.7%–1.3% for seeds incubated at 20, 25 and 30 ºC. When incubated for different durations in concentrated sulphuric acid, the germination percentage of normal seedlings ranged from 41%–100% (Table 1). Germination of nicked seeds ranged from 95%–97%. No radicle emergence and hence no seedlings were observed from seeds treated with organic solvents, oven-dry-heat treated seeds, or water treated seeds. Germination of seeds treated with sulphuric acid varied by duration of treatment and differed from germination of the untreated control seeds. Seeds incubated for 20 and 30 minutes in sulphuric acid showed higher germination than untreated controls, nicked seeds, and seeds incubated for 10 minutes in sulphuric acid (Table 1).

Seedling length of untreated control seeds incubated at 25 ºC was significantly greater than from seeds incubated at 20 and 30 ºC. Seedling lengths were similar for seeds treated with concen-

trated sulphuric acid for 20 and 30 min and incubated at 20 and 25 ºC. Seedling lengths of nicked seeds were similar when incubated at 25 and 30 ºC. Seeds treated with sulphuric acid for 20 and 30 min and incubated at 30 ºC showed highest overall seedling length (Fig. 1).

Table 1. Germination percentage of normal seedlings of untreated control seeds of *A. chinensis* and normal seedlings

Incubation temperature	Germination (%)				
	Control	Duration of treatment in H_2SO_4			Nicking
		10 min	20 min	30 min	
20°C	0.66 (±0.54)	40.67 (±3.31)	99.33 (±0.54)	99.99 (±0.00)	96.66 (±2.00)
25°C	1.33 (±0.54)	43.99 (±3.40)	99.33 (±0.54)	98.33 (±0.54)	95.33 (±1.10)
30°C	1.33 (±0.54)	43.33 (±1.44)	96.66 (±0.54)	98.66 (±1.10)	96.66 (±2.00)

Notes: The seeds weretreated with sulphuric acid (10, 20, 30 minutes) and nicking, incubated at different temperatures, evaluated after 21 days of incubation [lsd (p=0.05) = 1.77; ± = SEM].

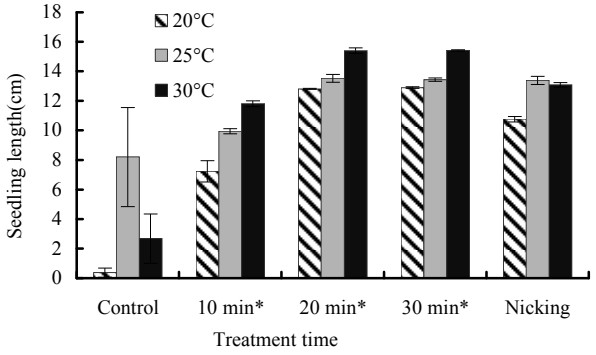

Fig. 1 Seedling length of *A. chinensis* treated for 10–30 min in concentrated sulphuric acid (*) and nicking, incubated at 20, 25 and 30ºC [lsd (p=0.05) = 2.50]

Seedling dry weight was lowest for untreated control seeds at all three incubation temperatures, but other treatments yielded significantly higher seedling weights. Seedling dry weights from seeds treated with sulphuric acid for 20 and 30 min were similar at all three incubation temperatures, but were significantly higher than weights of seedlings from seeds incubated for 10 minutes in sulphuric acid and control seeds at all incubation temperatures, and nicked seeds at 25 and 30 ºC incubation temperatures. The seedling dry weights from nicked seeds at 20 ºC were highest, not only in comparison with those from nicked seeds incubated at 25 and 30 ºC, but also in comparison with those from seeds treated in sulphuric acid for any duration and at any incubation temperature (Fig. 2).

G_{RI} and G_V yielded trends similar to those for seedling weight, but the highest indices resulted from incubation at 25 ºC and 30 ºC for 30 min with concentrated sulphuric acid (Figs. 3, 4, 5).

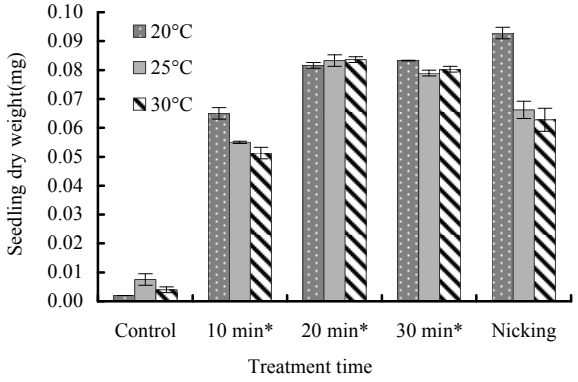

Fig. 2 Seedling dry weight from germinated seeds of *A. chinensis* treated for 10–30 min in concentrated sulphuric acid (*) and nicking, incubated at 20, 25 and 30ºC [lsd (p=0.05) = 0.01]

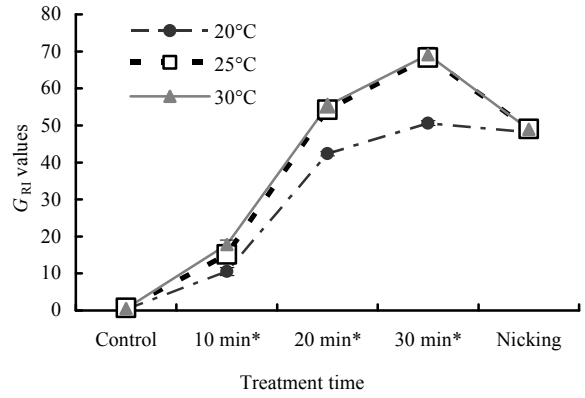

Fig. 3 G_{RI} values from germinated seeds of *A. chinensis* treated for 10–30 min in concentrated sulphuric acid (*) and nicking, incubated at 20, 25 and 30ºC [lsd (p=0.05) = 6.03]

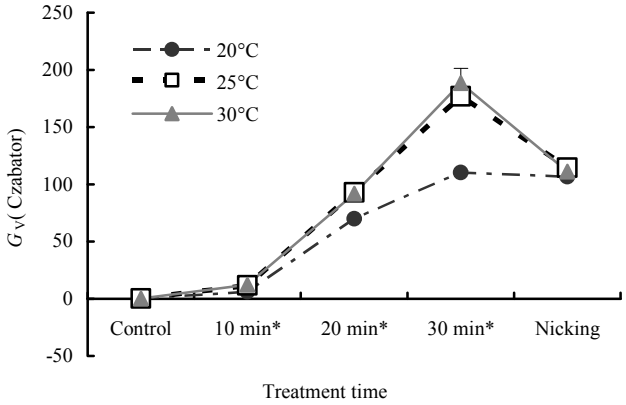

Fig. 4 G_V (Czabator) of germinated seeds of *A. chinensis* treated for 10–30 min in concentrated sulphuric acid (*) and nicking[lsd (p=0.05) = 23.53]

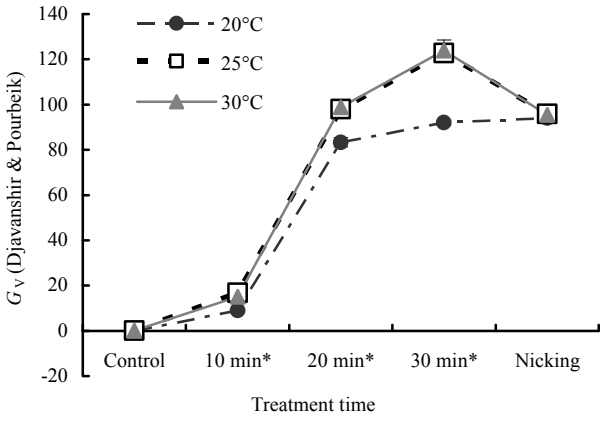

Fig. 5 G_V (Djavanshir & Pourbeik) of germinated seeds of *A. chinensis* treated for 10–30 min in concentrated sulphuric acid (*) and nicking, incubated at 20, 25 and 30ºC [lsd (p=0.05) = 9.99]

Discussion

The seeds of *A. chinensis* germinate at low rates in the wild due to their impervious, thick, and leathery seed coat. From the present investigation it is evident that seeds respond positively to pre-treatment, which increases germinability at temperatures typical of its geographic and altitudinal ranges (20 to 30ºC). In the wild, *A. chinesis* fails to produce enough seedlings to compensate for human exploitation despite the fact that pods and seeds are produced in abundance on the mother trees. Germination of seeds of many species of *Albizia* increases in response to pre-treatments, including hot or boiling water, oven-dry-heat, physical scarification, acid scarification, soaking in water, gibberellic acid, potassium nitrate and others (Msanga and Maghembe 1986; Sanjeevukumar et al. 1995; Kannan et al. 1996; Tigabu and Oden 2001; Alamgir and Hossain 2005; Ajiboye et al. 2009, 2011; Azad et al. 2010; Merou et al. 2011; Azad et al. 2012).

Most Leguminosae species have hard seed coats that are impervious to water and oxygen uptake (Justice and Bass 1979). The presence of a micropylar plug (Dell 1980) can delay the germination of the *A. lophanta*, but in many other species, delayed germination is attributed to the impermeability of the seed coat. Our objective in seed treatment was to ensure that maximum germination was achieved from a particular treatment with minimal or no damage to the seeds or to the developing seedlings. In the present investigation, treatment with water, ethyl alcohol, acetone, petroleum ether, oven-dry-treatment at 30, 40 and 60 ºC for 24 hours failed to relieve dormancy. Dormancy was relieved only in seeds treated with concentrated sulphuric acid and nicking. Cumulative germination and seedling length were greater for seeds incubated in sulphuric acid than for untreated controls, and best for seeds treated for 20 and 30 min and incubated at 30 ºC. Seedling lengths of nicked seeds incubated at all temperatures also exceeded those of control seeds. Seedling dry weights from seeds treated with sulphuric acid for 20 and 30 min were greater than those of all untreated controls at all incubation temperatures. G_{RI} and G_V were highest at 30 ºC. The reliability of G_{RI} as an index of seed germination in tree species was reported by Villagra 1995 and Kharlukhi et al. 2007. Higher G_{RI} indicates higher germination percentage. Lower G_{RI} values were recorded for seeds treated with sulphuric acid for 10 minutes, nicked seeds, and untreated control seeds, in which radicle emergence was spread over more than 14 days. Seeds treated for 20 or 30 min in sulphuric acid and incubated at 30 ºC showed complete radicle emergence within 6 days. G_V is a combination of completeness and speed of germination, higher values indicating faster and higher germination rates (Czabator 1962). The applicability of G_V in assessing germination was reported for *Cinnamomun camphora* (Chien and Lin 1994), three ash species (Piotto 1995), *Albizia procera* (Sanjeevukumar et al. 1995) and *Pinus kesiya* (Kharlukhi et al. 2007). The applicability of G_V proposed by Djavanshir and Porveik (1976) was also reported for *Aesculus indica* (Majeed et al.2010). Published results confirm that seeds of many species of *Albizia* germinate at higher rates after pre-treatment and this varies by species. While sulphuric acid treatment is useful for some species, the duration of incubation in sulphuric acid varies by species (Sanjeevukumar et al. 1995; Azad et al. 2012; Merou et al. 2011)

We recommend treatment of seeds for 20–30 min in concentrated sulphuric acid because it is more uniform and less labor intensive than nicking. The main disadvantage of nicking is that it is time consuming and labor intensive. Sulphuric acid treatment is less time-consuming and the acid is reusable. Personnel must, however, be careful when handling acids.

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